FORM PTO-1390 (REV 1-98) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER: 22681-0002

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U S APPLICATION NO (If known, see 37 CFR 1 5): 09/171081

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB97/01007	April 11, 1997	April 12, 1996

TITLE OF INVENTION
PROCESS FOR THE PREPARATION OF CLAVULANIC ACID

APPLICANT(S) FOR DO/EO/US
Sasa KRANJC et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [XX] This is a FIRST submission of items concerning a filing under 35 U S.C. 371.
2. [ ] This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This express request to begin national examination procedures (35 U.S C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [XX] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [XX] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau)
   b. [X] has been transmitted by the International Bureau
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US)
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). [unsigned]
10. [ ] A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3 31 is included.
13. [XX] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [ ] Other items or information:

Express Mail Label No. EM538745271US
Mailed October 13, 1998
[October 12, 1998 being a Federal holiday]

U.S. APPLICATION NO (if known, see 37 CFR 1 5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
	PCT/GB97/01007	22681-0002

17. [X] The following fees are submitted:	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1 492 (a) (1) - (5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1070.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $930.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $790.00		
International preliminary examination fee (37 CFR 1 482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) $720.00		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) $98 00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 930	
Surcharge of $130 00 for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	- 20 =		x $22 00	$	
Independent claims	- 3 =		x $82.00	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 930	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) +				$	
SUBTOTAL =				$ 930	
Processing fee of $130.00 for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1 492(f)).				$	
TOTAL NATIONAL FEE =				$ 930	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$	
TOTAL FEES ENCLOSED =				$ 930	
				Amount to be refunded:	$
				charged:	$

a. [X] A check in the amount of $ 930 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No 08-1641. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1 137 (a) or (b)) must be filed and granted to restore the application to pending status

SEND ALL CORRESPONDENCE TO.

Heller Ehrman White & McAuliffe
525 University Avenue
Palo Alto, CA 94301

Derek P. Freyberg
SIGNATURE

Derek P. Freyberg
NAME

29,250
REGISTRATION NUMBER

Tel.: (650) 324-7014

PATENTS

Attorney Docket No. 22681-0002

EXPRESS MAIL LABEL INFORMATION - 37 CFR 1.10

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Mailed October 13, 1998 [October 12, 1998 being a Federal holiday]

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Sasa KRANJC et al. :

App. No.: (not yet known) : Art Unit: (not yet known)
Int'l App. No. PCT/GB97/01007

Filed: (herewith) : Examiner: (not yet known)
Int'l Filing Date: April 11, 1997

For: PROCESS FOR THE PREPARATION OF CLAVULANIC ACID

Box PCT
Assistant Commissioner for Patents
Washington, DC 20231

Sir:

PRELIMINARY AMENDMENT

Please amend the above-identified application , before examination, as follows:

In the Specification:

Add an abstract, as follows:

--ABSTRACT OF THE DISCLOSURE

A process for preparation of clavulanic acid includes fermentation of a clavulanic acid-producing species of Streptomyces in a fermentation broth containing sources of assimilable carbon, nitrogen, and phosphorus, where the

concentration of assimilable phosphorus in the fermentation broth is less than 0.15% w/v. --

In the Claims:

Cancel Claims 1-14.

Add Claims 15-34 as follows:

-- 15. A process for production of clavulanic acid comprising fermentation of a clavulanic acid-producing species of Streptomyces in a fermentation broth containing sources of assimilable carbon, nitrogen, and phosphorus,
wherein the starting concentration of assimilable nitrogen in the fermentation broth is greater than 5%, and
wherein the concentration of assimilable phosphorus in the fermentation broth is less than 0.15% w/v during a growth phase of the fermentation and is allowed to decrease after cessation of the growth phase.

16. A process as claimed in Claim 15, wherein the concentration of assimilable phosphorus is allowed to decrease after a fermentation time of 40 hours.

17. A process as claimed in Claim 16, wherein no assimilable phosphorus is added after a fermentation time of 40 hours.

18. A process as claimed in Claim 16 wherein the concentration of assimilable phosphorus up to a fermentation time of 40 hours is 0.0015% to 0.15% w/v.

19. A process as claimed in Claim 18, wherein no assimilable phosphorus is added after a fermentation time of 40 hours.

20. A process as claimed in Claim 18 wherein the concentration of assimilable phosphorus is 0.002% to 0.05% w/v.

21. A process as claimed in Claim 20, wherein no assimilable phosphorus is added after a fermentation time of 40 hours.

22. A process as claimed in Claim 15, wherein the source of assimilable phosphorus is sodium phosphate, potassium phosphate, sodium dihydrogen phosphate, potassium dihydrogen phosphate, disodium hydrogen phosphate, dipotassium hydrogen phosphate, or a mixture thereof.

23. A process as claimed in Claim 15, wherein ammonia is not the sole source of assimilable nitrogen.

24. A process as claimed in Claim 23, wherein the source of assimilable nitrogen does not include ammonia.

25. A process as claimed in Claim 23, wherein the source of assimilable nitrogen includes soy bean flour.

26. A process as claimed in Claim 24, wherein the source of assimilable nitrogen includes soy bean flour.

27. A process as claimed in Claim 23, wherein the source of assimilable nitrogen includes ammonium sulphate.

28. A process as claimed in Claim 24, wherein the source of assimilable nitrogen includes ammonium sulphate.

29. A process as claimed in Claim 23, wherein a nitrogen-free compound is used to control pH.

30. A process as claimed in Claim 24, wherein a nitrogen-free compound is used to control pH.

31. A process as claimed in Claim 15, wherein the microorganism is Streptomyces clavuligerus, Streptomyces jumonjinensis, Streptomyces katsurahamanus, or Streptomyces sp. P6621.

32. A process as claimed in Claim 15, wherein the process is fed batch or continuous, with intermittent or continuous addition of a source of assimilable phosphorus.

33. A process as claimed in Claim 15, wherein the volume of the fermentation broth is greater than 10^4 liters.

34. A process as claimed in Claim 33, wherein the volume of the fermentation broth is 5×10^4 liters. --

REMARKS

The Amendment

Entry of this amendment is respectfully requested. No new matter is added by the amendments, because the new claims find support in the application as filed. In particular, the new claims limit the claim dependencies and rewrite the claims in more standard US form.

Claims 15-34 are in this application, Claims 1-14 having been canceled, and Claims 15-34 having been added by this amendment. Entry of the amendment and allowance of the claims are requested.

Respectfully submitted,

Derek P. Freyberg
Attorney for Applicants
Reg. No. 29,250

Heller Ehrman White & McAuliffe
525 University Avenue
Palo Alto, CA 94301-1900
(650) 324-7014
October 13, 1998

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59 Rec'd PCT/PTO 13 OCT 1998
PCT/GB97/01007
09/171081

PROCESS FOR THE PREPARATION OF CLAVULANIC ACID

This invention relates to a process for preparation of clavulanic acid.

Clavulanic acid is the common name for (2R, 5R, Z) - 30(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. Clavulanic acid and its alkali metal salts and esters are active as inhibitors of beta lactamase produced by some Gram positive as well as Gram negative micro-organisms. In addition to inhibition of beta lactamase, clavulanic acid and alkali metal salts thereof also have a synergistic action with penicillin and cephalosporin antibiotics. Clavulanic acid and its salts are used in pharmaceutical preparations to prevent the deactivation of beta lactam antibiotics. Commercial preparations contain potassium clavulanate in combination with amoxycillin trihydrate. Potassium clavulanate is more stable than the free acid or other salts.

Clavulanic acid is prepared by fermentation of micro-organisms such as strains of Streptomyces for example S.clavuligerus NRRL 3585, S.jumonjinensis NRRL 5741 and S.katsurahamanus IFO 13716 and Streptomyces sp.P6621 FERM P2804. The aqueous culture obtained after fermentation is purified and concentrated in accordance with conventional processes for example filtration and chromatographic purification as disclosed in GB 1508977, prior to extraction of the aqueous solution with an organic solvent to obtain a solution of impure clavulanic acid in the solvent.

WO95/23870 and WO96/28452 disclose improved commercial processes for purification of clavulanic acid and preparation of potassium clavulanate. Growth of an antibiotic producing micro-organism may include two phases; the growth phase during which biomass is produced and the subsequent stationary phase during which growth does not incur. Secondary metabolites such as antibiotics are usually produced during the stationary phase.

Fed batch fermentation processes are well known for

antibiotic production and have been preferred for production of clavulanic acid. Control and maintenance of desired levels of assimilable sources of nitrogen and carbon in the fermentation broth is well illustrated by Lee J S et al, Kor.Jour.Microbiol. 1978, Vol 15, No 1, p 21-29, which describes the improvement of the fermentation process for preparation of penicillin by control of addition of the assimilable nitrogen and carbon source according to the needs of microorganisms in the fermenter. Lilley G et al, J.Chem.Tech.Biotechnol. 1981, Vol 31, p 127-134 illustrates that the production of antibiotics by Streptomyces species can be controlled by changing of concentration of assimilable nitrogen and carbon source and source of phosphorus. For example, the production of thienamycin in the fermenter does not start until the concentration of phosphorus approaches zero. EP 82522 illustrates use of continuous or intermittent addition of the assimilable carbon source in fermentation of S.clavuligerus NRRL 3585. Regulation of the amount of ammonia is disclosed as WO96/18743. Continuous fermentation processes have not been disclosed for clavulanic acid manufacture.

According to the present invention a process for production of clavulanic acid comprises fermentation of a clavulanic acid producing species of Streptomyces in a fermentation broth containing assimilable sources of carbon and nitrogen, wherein the concentration of phosphorus in the fermentation broth is less than 0.15% w/v.

The phosphorus concentration is preferably maintained below a limit of 0.15% w/v during the growth phase after which the phosphorus concentration may be allowed to decrease. The growth phase for a typical clavulanic acid fermentation lasting for a total of 5 to 6 days may be complete by about 40 hours. The source of assimilable phosphorus may be present as a phosphate salt for example sodium or potassium phosphate, sodium or potassium dihydrogen phosphate or disodium or dipotassium hydrogen phosphate or mixtures thereof. The phosphorus concentration referred to in this specification is determined as the percentage w/v of phosphorus equivalent to

the amount of assimilable phosphorus compound present.

The phosphorus concentration is preferably 0.0015 to 0.15% w/v, more preferably 0.002 to 0.015% w/v. The phosphorus concentration is preferably allowed to reduce to a low value, preferably zero by the 40th hour of fermentation.

Regulation of the amount of assimilable phosphorus in accordance with the present invention may afford unexpectedly high yields of clavulanic acid.

The concentration of assimilable carbon source may be selected by routine trials dependent on the characteristics of the Streptomyces strain employed. The proportion of a carbon source such as glycerol, glycerol trioleate or corn starch in the starting medium may be higher than 5% w/v and further quantities of a carbon source may be added during the fermentation in accordance with usual fed batch procedures.

Assimilable nitrogen may be provided by proteinaceous matter in the starting media. Alternatively or in addition ammonia may be introduced into the fermenter. Ammonia has also been used to regulate the pH of the fermentation broth during the course of the fermentation. However we have found that use of ammonia both as the sole source of assimilable nitrogen and also for pH regulation is undesirable. A high concentration of ammonia can poison the microorganisms and a pH which is too low results in less effective clavulanic acid biosynthesis. Accordingly it is preferred that an assimilable source of nitrogen, for example soya bean flour, is added to the starting medium and that an ammonium salt such as ammonium sulphate is added during the course of the fermentation to provide further nitrogen as necessary.

According to a preferred aspect of the present invention a nitrogen free compound, preferably ammonium hydroxide is used to control pH. This results in a later decrease in the level of biomass than is the case if ammonia is used as the sole nitrogen source and pH regulator. The results of a comparison with a classic fermentation are shown in Figure 1. The viscosity, which is proportional to the amount of the biomass is shown for a classical fermentation (broken line) and for a

fermentation in accordance with the invention (solid line).

The assimilable nitrogen source may be flour, for example soya flour or cotton seed flour. The amount of assimilable nitrogen is preferably 0.5 to 15% w/v, more preferably 1.5 to 7.5% w/v. The amount of nitrogen is advantageously greater than 5%.

The invention relates particularly to fermentation of the Streptomyces species S.clavuligerus NRRL 3585, S.jumonjinensis NRRL 5741 and S.katsurahamanus IFO 13716 and particularly Streptomyces sp.P6621 FERM P2804. The invention yield improved yields of clavulanic acid from S.clavuligerus The invention finds particular application in relation to commercial scale fermentations particularly but not exceeding broth volumes of 10^4 l, preferably 5 x 10^4 l

The fermentation broth may be treated as disclosed in our WO95/23870 or by other known methods by which potassium clavulanate of high purity may be prepared.

The invention is further described by means of example but not in any limitative sense.

EXAMPLE 1

A) CULTIVATION OF STREPTOMYCES SP.P 6621 FERM P 2804

Strain selection and maintenance

The most productive clones of Streptomyces sp. PP 6621 FERM P 2804 were obtained by selection methods. The most productive cultures of this microorganism were stored and were further used as a source for new selection cycles.

A colony of Streptomyces sp. PP 6621 FERM P 2804 was aseptically transferred in to a sterile potter with sterile water ($2cm^3$) and homogenised. Fragments of the mycelium were transferred onto an agar slope and incubated to maturity (for 10 to 14 days) in a thermostat at 25°C.

After 8 to 10 days the agar surface was overgrown by a grey-green bacterial mycelium. Spores were scraped from the surface, aseptically inoculated into a seed vegetative medium and incubated on a shaker for 24 h at 250 rpm and at 25°±1°C.

Homogeneous suspension of spores from agar slopes may be stored in skimmed milk (which can be used as a protective medium) for more than two months.

After completion of the vegetative stage, part of the culture was aseptically transferred to a fermentation medium and was incubated on a rotary shaker for 96 h. After the finished fermentation state the content of clavulanic acid was analysed. Cultures which gave the highest yields were used as laboratory inoculum in the fermenter.

The entire procedure was carried out under aseptic conditions.

Strains may be stored on slope agar at 4°C maximum for 4 weeks, in skimmed milk in the same condition for 2 months and lyophilised strains may be stored at 4°C for a period of years.

Composition of media for selection of strain for inoculation in the fermenter

Media for slopes and Petri dishes

Composition	amount
dextrin	10 g
KH_2PO_4	1 g
$MgSO_4$ $7H_2O$	1 g
NaCl	1 g
$(NH_4)_2SO_4$	1 g
$CaCO_3$	4 g
Trace elements *	1 cm^3
agar	20 g
demineralised water	to 1000 cm^3

The composition was prepared in accordance with classical methods.

Trace elements*

Composition	amounts
$CaCl_2$ $2H_2O$	10.0 g
$MgCl_2$ $6H_2O$	10.0 g
NaCl	10.0 g
$FeCl_3$ $6H_2O$	3.0 g
$ZnCl_2$	0.5 g
$CuCl_2$ $2H_2O$	0.5 g
$MnSO_4$ H_2O	0.5 g
demineralised water	to 1000 cm^3

Vegetative media for strain selection

Composition	amounts
corn starch	10.0 g
soybean flour	20.0 g
KH_2PO_4	0.6 g
Estol (Priolube 1435)	5.0 g
tap water	to 1000 cm^3

Fermentation media for strain selection

Composition	amounts
corn starch	9.6 g
soybean flour	38.5 g
KH_2PO_4	1.2 g
Estol (Priolube 1435)	23.0 g
glycerol	5.0 g
morpholine propane sulphonic acid	12.0 g
trace elements*	10.0 ml
tap water	to 1000 ml

Preparation of the laboratory inoculum

The origin of culture for preparation of laboratory inocula was cultured from an agar slope. The chosen slope agar was filled aseptically with sterile water ($10cm^3$), spores were scraped off and homogenised in a sterile potter. The solution of spores was used as a laboratory inoculum.

VEGETATIVE PHASE IN PRE-SEED TANK

Media for pre-seed tank

Volume of pre-seed = 500l

Volume of medium = 350l

Composition	amounts
corn starch	7.0 kg
soybean flour	7.0 g
NaH_2PO_4	0.185 kg
Estol (Priolube 1435)*	0.7 kg
synperonic	0.150 kg
tap water	to 350 l

*soybean oil can be used instead of estol.

The inoculum was transferred in a medium that had been sterilised in pre-seed tank and cooled by sterile air to 28°C. The vegetative phase lasted from 50 to 70 hours at a temperature 28°±1°C, pressure 0.3 Bar and with aeration using sterile air and with consistent mixtures.

Parameters of growth in pre-seed tank

time/h (h)	pH	PMV%	decolourisation/min
0	7.20	--	--
4	7.25	10	> 5
10	7.35	8	> 5
16	7.30	10	> 5
22	7.20	16	4
28	7.02	17	2.5
34	6.85	18	0.5
39	6.66	20	0.3
45	6.60	21	0.5
51	6.52	22	1.0
56	6.39	22	1.0
61	6.45	20	1.3

Legend:
pH = pH value of sample
PMV% = volume % of culture in sample
decolourisation = time necessary for decolourisation of methylene dye

VEGETATIVE PHASE IN SEED FERMENTER

Media for seed fermenter
vol. of seed fermenter = 7500 l
vol. of media = 4500 l

Composition	amount
corn starch	90 kg
soybean flour	90 kg
NaH_2PO_4	2.4 kg
Estol (Priolube 1435)*	9 kg
Synperonic	0.5 kg
water	to 4500 l

* Soybean oil can be used instead of estol.

The vegetative phase from the pre-seed tank was transferred under pressure into a medium that had been sterilised in the seed fermenter and cooled by sterile air to 28°C. Air was sterilised by filters with a pore size of 0.2 μm.

The vegetative phase lasted from 10 to 20 h at 28°±1°C, pressure 0.3 Bar, aeration by sterile air and constant mixing.

Growth was monitoring by analysis of pH, PMV%, decolourisation of methylene and by microscopic examination of samples.

Parameters of growth in pre-seed tank

Time/h	pH	PMV%	declourisation/min
0	7.20	--	--
6	7.10	15	> 5
12	6.87	20	1.5
16	6.65	22	0.3

Legend:
pH = pH value of sample
PMV% = volume % of culture in sample
decolourisation = time necessary for decolourisation of methylene dye

B) BATCH FERMENTATION OF STREPTOMYCES SP. P 6621 FERM P 2804 IN FERMENTER

Media for fermenters
Vol. of fermenter = 90 000 l
Vol of media = 60 000 l

Composition	amount
corn starch	570 kg
soybean flour	2300 kg
NaCl	6 kg
Estol (Priolube 1435)*	1680 kg
NaH_2PO_4	5 kg
$MgCl_2$ $6H_2O$	7 kg
$FeCl_3$ $6H_2O$	1.6 kg
$ZnCl_2$	0.5 kg
$MnSO_4$ H_2O	0.1 kg
Synperonic	25 kg
water	to 60 m^3

Legend:
- Estol is a generic name for glycerol trioleate; (Priolube 1435 registered trade mark of Unichem GmbH, Germany)
- Synperonic (registered trade mark of ICI GB) is a propylenglycol antifoam agent
* Soybean oil can be used instead of Estol.

4700 l of a culture of Streptomyces sp. PP 6621 FERM P 2804 in the vegetative phase of growth from the seed fermenter was inoculated by a sterile transfer into a sterile starting medium (60 000l) in a 90 000 l stainless steel fermenter equipped for mixing and a delivery of sterile air through filters with a 0.2 μm pore size. The fermentation media and all inlet-pipes were sterilised and cooled by sterile air to 24°C. The fermentation phase from seed fermenter was maintained at 24°C - 25°C and 0.3 Bar. The broth was mixed and aerated during the course of whole fermentation and the pH of the media was maintained by addition of 25% aqueous solution of ammonium hydroxide at value 6.8 - 6.9. The fermentation lasted for 96h and the resultant concentration of clavulanic acid was 3580 mg/l.

During the course of the fermentation of Streptomyces sp.

PP 6621 FERM P 2804 a source of phosphorus and an assimilable source of nitrogen (500 kg of soybean flour in 5000 l of water and 25% aqueous solution of ammonium hydroxide) were added as follows:

Concentration of assimilable sources of phosphorus and nitrogen in the fermentation broth

time	phosphorus concentration (% w/v)	nitrogen concentrate (% w/v)
0	0,035	1,73975
8	0,030625	1,692286
16	0,0095	1,331
24	0.005188	0,9785
32	0,004638	0,5527
40	0,003638	0,69945
48	0,000863	0,9128
56	0,000863	0,8475
64	0	0,709
72	0	0,653625
80	0	0,571
88	0	0,47675
96	0	0,53825
104	0	0,7555
112	0	0,77025
120	0	0,673375
128	0	0,78725
136	0	0,734625
144	0	0,8985

The concentration of phosphorus after the 51st hour of the fermentation was below the detection limit.

The pH value reached in the first hours of the culture growth rose to almost 7.5. During this time phosphorus was consumed and clavulanic acid started to be produced, because of this the pH decreased and control of the pH of the media was

necessary to maintain the level of pH at the optimum value.

EXAMPLE 2

PROLONGATION OF THE VEGETATIVE PHASE OF FERMENTATION BY USE OF AMMONIUM SULPHATE AS ASSIMILABLE SOURCE OF NITROGEN AND SODIUM HYDROXIDE AS REGULATOR OF PH

A medium used with the same proportions of ingredients as Example 1B was placed in two stainless steel fermenters (500l each). The fermentation in the first fermenter was run under the same conditions as were described in the Example 1B. The fermentation conditions in second fermenter differed from that described in Example 1B only in that an 11% aqueous solution of ammonium sulphate at 9 cm^3/l was added to the fermentation broth during the period between the 40th and 60th hours following inoculation. The pH was maintained on the desired level by sodium hydroxide. After the 60th hour we stopped the addition of nitrogen source was stopped. The viscosity of the fermentation broth, which is proportional to the amount of biomass, was analysed during the course of fermentation.

Time	Run 1, viscosity (m Pa.s)	Run 2, viscosity (m Pa.s)
0	/	/
8	/	/
26	474	551
44	728	714
62	948	998
80	995	1076
98	936	1226
116	824	863
128	628	873

CLAIMS

1. A process for production of clavulanic acid comprising fermentation of a clavulanic acid producing species of Streptomyces in a fermentation broth containing assimilable sources of carbon and nitrogen, wherein the starting concentration of assimilable nitrogen is greater than 5% and, wherein the concentration of assimilable phosphorus in the fermentation broth is less than 0.15% w/v during the growth phase the phosphorus concentration being allowed to decrease after cessation of the growth phase.

2. A process as claimed in claim 1, wherein the phosphorus concentration is allowed to decrease after a fermentation time of 40 hours.

3. A process as claimed in any preceding claim, wherein the phosphorus concentration up to a fermentation time of 40 hours is 0.0015 to 0.15% w/v.

4. A process as claimed in claim 3, wherein the said phosphorus concentration is 0.002 to 0.05% w/v.

5. A process as claimed in any preceding claim, wherein no assimilable phosphorus is added after a fermentation time of 40 hours.

6. A process a claimed in any preceding claim, wherein the source of assimilable nitrogen does not include ammonia.

7. A process as claimed in claim 6, wherein the sole source of assimilable nitrogen is not ammonia.

8. A process as claimed in any preceding claim, wherein the source of assimilable nitrogen is flour.

9. A process as claimed in any preceding claim,

wherein the assimilable nitrogen source is ammonium sulphate.

10. A process as claimed in any preceding claim, wherein the concentration of the assimilable nitrogen source is 0.5 to 15% w/v.

11. A process as claimed in any preceding claim, wherein the source of assimilable phosphorus is sodium or potassium phosphate, sodium or potassium dihydrogen phosphate or disodium or dipotassium hydrogen phosphate or a mixture thereof.

12. A process as claimed in any preceding claim, wherein the microorganism is Streptomyces clavuligerus, Streptomyces jumonjinensis, Streptomyces katsurahamanus or Streptomyces sp. P6621.

13. A process as claimed in any preceding claim, wherein the process is fed batch or continuous with intermittent or continuous addition of an assimilable source of phosphorus.

14. A process as claimed in any preceding claim, wherein the volume of the fermentation broth is greater than 10^4 l.

15. A process as claimed in claim 14, wherein the volume is 5×10^4 l.

Please type a plus sign (+) inside this box → ☐

PTO/SB/01 (12-97)
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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☐ Declaration Submitted with Initial Filing OR ☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	22681-0002
First Named Inventor	Sasa KRANJC
COMPLETE IF KNOWN	
Application Number	09/171,081
Filing Date	
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PROCESS FOR THE PREPARATION OF CLAVULANIC ACID

(Title of the Invention)

the specification of which
☐ is attached hereto
OR
☒ was filed on (MM/DD/YYYY) 04/11/1997 as United States Application Number or PCT International Application Number PCT/GB97/01007 and was amended on (MM/DD/YYYY) _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	Certified Copy Attached? NO
P-9600120	Slovenia	04/12/1996	☐	☐	☒
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)

☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

Burden Hour Statement: This form is estimated to take 0.4 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

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DECLARATION — Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number *(if applicable)*

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: ☐ Customer Number → *Place Customer Number Bar Code Label here*
OR
☒ Registered practitioner(s) name/registration number listed below

Name	Registration Number	Name	Registration Number
Derek P. Freyberg	~~29250~~	Y. Ping Chow	30,740
William B. Anderson	P-41,585	Walter Kurz	37,373
Edward J. Lynch	24,422	Herwig von Morze	~~29,484~~

☐ Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto.

Direct all correspondence to: ☐ Customer Number or Bar Code Label ____ *OR* ☒ Correspondence address below

Name	~~Derek P. Freyberg~~				
Address	Heller Ehrman White & McAuliffe				
Address	525 University Avenue				
City	Palo Alto,	State	CA	ZIP	94301-1900
Country	U.S.A.	Telephone	(650) 324-7014	Fax	(650) 324-0638

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
Sasa	KRANJC

Inventor's Signature	Kranjc SX					Date	Nov. 23 1998
Residence: City	Ljubljana	State		Country	Slovenia	Citizenship	SI
Post Office Address	Trzaska 51a						
Post Office Address	(Ljubljana)						
City	Ljubljana	State		ZIP	1000	Country	Slovenia

☒ Additional inventors are being named on the 1 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

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DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 1 of 1

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
Artur	RACMAN

Inventor's Signature						Date	Nov. 23 1998
Residence: City	Crnelovei-Murska Sobota	State	SLX	Country	Slovenia	Citizenship	SI
Post Office Address	Dalmatinova 21						
Post Office Address	(Crnelovei-Murska Sobota)						
City	Crnelovei-Murska Sobota	State		ZIP	9000	Country	Slovenia

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname

Inventor's Signature						Date	
Residence: City		State		Country		Citizenship	
Post Office Address							
Post Office Address							
City		State		ZIP		Country	

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname

Inventor's Signature						Date	
Residence: City		State		Country		Citizenship	
Post Office Address							
Post Office Address							
City		State		ZIP		Country	

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